UNIVERSITY OF PENNSYLVANIA

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MARGARET SUMWALT

A THESIS

IN PHYSIOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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POTENTIAL DIFFERENCES ACROSS THE CHORION OF THE *FUNDULUS* EGG.

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Loeb and Cattell (16) in 1915 reported the results of certain experiments on *Fundulus* eggs, which they did not satisfactorily explain. The central observation was that when KCl had penetrated the egg in sufficient amount to stop the heart of the embryo, it could escape, effecting recovery of the heart beat, if the eggs were placed in a solution of some other salt or in a dilute solution of acid, but not if they were placed in distilled water. An analogous observation on very different material was made ten years later by Michaelis and Fujita (20, 21), who found that K and other cations will pass through apple skin and dried collodion membranes into salt solution, but not into distilled water. Their explanation was that these membranes are permeable for cations but not for anions; when it is possible for cations on the two sides of such a membrane to be exchanged, movement of cations across the membrane can occur, but not otherwise. This explanation Michaelis, with several collaborators, has supported by abundant electrical and chemical evidence (5, 20–26).

In the present experiments some of the electrical methods used by Michaelis and his collaborators in the study of apple skin and artificial membranes have been applied to the chorion of the *Fundulus* egg. The results obtained indicate that the electrical properties of this membrane are similar in many respects to those of dried collodion membranes. If they are interpreted analogously, a partial explanation of the results of Loeb and Cattell is afforded.

* I wish to express my indebtedness and gratitude to Dr. M. H. Jacobs who suggested this application of electrical methods to the study of the permeability of the *Fundulus* egg, and to Dr. William R. Amberson with whom the problem was at first prosecuted jointly. Part of the work was done during my tenure of a Dean Van Meter Alumnæ Fellowship from Goucher College.

Material and Method.

The *Fundulus* embryo is enclosed by two membranes: the chorion, a non-cellular, tough, but elastic shell, and the ectoderm of the embryo. In the experiments to be reported, potential differences were measured across the chorion only. It is hoped, however, that a similar study of the ectoderm may be made in the near future.

The properties of the chorion do not appear to vary greatly with the age of the egg, except for a slight decrease in elasticity with time. Fertilized eggs of any convenient age could therefore be used. Most of those employed in these experiments were between 5 and 9 days old at a season when hatching occurred between the 10th and 13th day. A few experiments were performed with satisfactory results on unhatched cold storage eggs 28 days old.

Potential differences were measured between the inside and the outside of the chorion of single eggs. The subchorionic fluid surrounding the embryo constituted a constant environment for the inner surface of the membrane, while solutions of various compositions and concentrations were applied to the outside. In an ideal system, successive applications of two different solutions to the outside of a membrane should produce a change in the membrane potential equal to the P.D. which would be observed if the membrane were placed between the solutions in question. Though the egg is not an ideal system, potential differences arrived at by this method are probably not greatly in error. Measurements across apple skin are, of course, subject to the same disadvantage, but in the hands of Fujita (5) have, by the use of the method here employed, yielded significant results.

To make the inside electrical contact, a capillary pipette was inserted into an egg so that its orifice lay in the subchorionic fluid between the embryo and the chorion. The pipette was filled with saturated KCl and communicated with a saturated KCl calomel half cell. Outside contact was made by dipping a like half cell into the solution in which the egg was immersed. The arrangement of the apparatus is shown schematically in Fig. 1.

The two electrodes were connected into a simple potentiometer circuit. A Leeds and Northrup student potentiometer was used, and the null instrument was a d'Arsonval galvanometer of high

sensitivity. The electrical resistance of the system without an egg was from 10,000 to 100,000 ohms, according to the diameter of the pipette and concentration of the solution into which the electrodes dipped. With an egg on the pipette, the resistance was still higher. Since the galvanometer deflections diminished as the resistance increased, readings were more accurate when the egg lay in concentrated solutions than in dilute. The sensitivity of the galvanometer was sufficient to give a deflection of at least 1 mm. for 5 millivolts with an egg on the pipette in M/20,000 KCl. In this, the most dilute solution used in any of the experiments, the P.D. was sufficiently large to render the experimental error reasonably small (about 5 per cent.). In M/2,000 KCl the galvanometer deflections were of the order of 1 mm. for 1 millivolt, so that considerable accuracy was possible in the determinations at this and greater concentrations.

So high was the resistance in the circuit when an egg was impaled on the electrode that, because of the humid weather conditions prevailing at Woods Hole, and the presence in the laboratories of traces of salts from the sea water, none of the precautions originally used to shield the apparatus prevented short circuit leaks. The work must have been abandoned had not independence of weather conditions been finally secured in a dry room.[1] Here no leaks occurred so long as door and windows were kept closed.

When Osterhout, Damon, and Jacques (28) measured P.D. in *Valonia,* they immersed the cells only partly in the experimental solution, and tested for short circuits at the hole where the pipette entered the cell by comparing the values for part immersion with others obtained when the cell was completely submerged in the same experimental solution. The presence of a leak was shown by diminished P.D. in the latter case.

The *Fundulus* egg, however, is too small for partial immersion without danger of complete wetting by capillarity. In the present study, therefore, an egg was completely immersed in the experimental solutions throughout a determination. That little or no leakage occurs ordinarily under these conditions is indicated by

[1] This was a room in which there was no running water, which had never been used for any experimentation, and which had been closely shut up. Solutions were prepared elsewhere, and the area of free water surfaces was reduced to a minimum.

the constancy and reproducibility of the P.D.'s obtained. After puncturing an egg in sea water, successive washings in the first experimental solution usually gave steadily ascending P.D. values until a definite maximum was obtained, and this maximum was reproducible within certain limits which will be mentioned later. But, occasionally, the wound failed to close tightly around an entering pipette, and in such a case the observed P.D.'s were small and erratic. This behavior occurred with large pipettes and with pipettes improperly shaped for making a clean puncture, and was more frequent with older eggs in which the chorion was less elastic. Failure to obtain a tight seal about the electrode could often be detected by the visible escape of subchorionic fluid. But the presence of even an invisible leak was recognizable by the inconstancy of the observed P.D. Results on leaky eggs were always discarded.

The difference of potential between the electrodes alone, dipping directly into an experimental solution, amounted at times to 2 or 3 millivolts, but it was reproducible no matter what the dilution or composition of the solution, so long as sufficient pressure was maintained to keep a gentle stream of KCl issuing from the mouth of the pipette electrode. The density of the saturated KCl made this flowing junction visible. If the pressure dropped to zero, however, so that the visible flow of KCl ceased, anomalous P.D.'s were observed whose magnitude increased with the dilution of the solution surrounding the electrode tips. In the most dilute solutions used, these sometimes attained a magnitude of 100 millivolts or more. The site of these P.D.'s was the mouth of the pipette electrode, as was shown by short circuiting [2] it; and the cause, at least in part, its small size. Pairs of large tubes showed no such effect. Agar-filled tips of unequal size showed them even more markedly. All of the data presented in this paper have been

[2] The pipette electrode was short circuited as follows: As Fig. 1 shows, the pipette is not the only avenue of contact with its calomel half cell. There is also a communication with that half cell through a siphon dipping into a reservoir. When the P.D. between the calomel half cells was to be measured, free from the influence of the P.D. occurring at the mouth of the pipette, the experimental solution was placed in some vessel other than the egg chamber. Into this dipped the calomel half cell which usually made contact with the solution in which an egg was immersed; and the other half cell was put in contact with it through the siphon.

corrected for the electrode potential measured just before or just after each egg determination, with the electrodes dipping into sea water and a flowing junction at the mouth of the pipette electrode.

Although a flowing junction could be maintained between the pipette and the experimental solution during preliminary tests of the electrodes, such a junction was impossible between the pipette and the subchorionic fluid of an egg. In fact, to prevent contamination of the egg contents with saturated KCl, a pressure was maintained in the capillary which, though sufficient to produce a flowing junction in open solution, permitted a slight ascent of egg substance into the capillary when balanced against the turgor of the egg. Two considerations, however, support the belief that the experimental data are free from artefacts produced by the electrodes. First, the tests of the electrodes alone show that high anomalous P.D. values were due to the pipette and appeared only when it was in dilute solutions. During a measurement of P.D. across the chorion the pipette was in subchorionic fluid, the concentration of which was of the same order as that of sea water. The pipette was thus protected by the egg against the environment in which the high P.D. at its mouth was produced. Second, the subchorionic fluid—KCl junction in the pipette was constant throughout an experiment. If there was a P.D. at this junction, the effect which it had disappears when *differences* between observed P.D.'s are considered; and this is the case with all the results given.

Pressure control in the pipette electrode was desirable for the two reasons already discussed: to maintain a flowing junction during the preliminary tests of the electrodes; and to prevent a flowing junction during egg measurements. Therefore an apparatus patterned after that used by Landis (7) for capillary injections was used. (See Fig. 1.) A Luer syringe communicating with the pipette half cell system made small sudden changes of pressure possible, and a reservoir in communication with the system through a siphon at another point maintained a constant head of pressure, the influence of which could be controlled by a stop cock.

The pipette communicated with the pressure control through a coil of hard rubber tubing sufficiently flexible to permit control

of the movement of the pipette with a Chambers micromanipulator. The egg lay in a chamber on the stage of a microscope, and the position of the pipette within it could be observed at all times during the course of the experiments.

The inside diameters of the capillary electrodes used were about 70 μ. In experiments with eggs, no systematic variation in P.D. was observed with pipettes of different sizes, except when so large a one was used that the chorion failed to close tightly around it. The puncture of an egg was always carried out in sea water in order to avoid carrying into it excess KCl on the outside of the pipette.

As a precaution against contamination of the experimental solution by diffusion of saturated KCl from the outside electrode, the electrode dipped not directly into the egg chamber, but into a thistle tube communicating with the egg chamber through 7 or

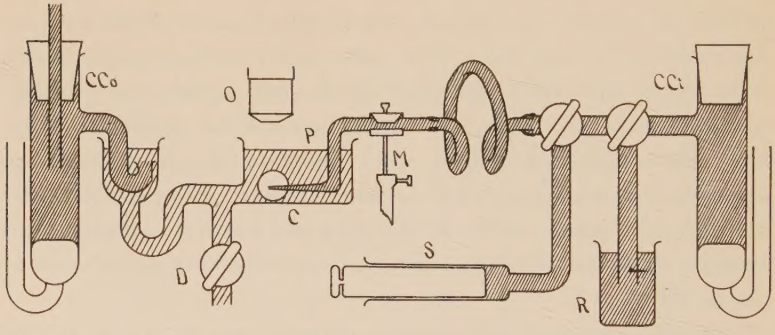

FIG. 1. Diagram of apparatus. The egg, impaled on the capillary pipette *P*, lay in a chamber *C* beneath the microscope objective *O*. Outside electrical contact was made through a calomel half cell *CCo* which dipped into the solution in which the egg was immersed at some distance from the egg, and drainage of the chamber was effected through the tube *D* at an intermediate point. The movements of the pipette were controlled by a Chambers micromanipulator *M*. The pipette is in communication through a coil of hard rubber tubing not only with the other calomel half cell *CCi*, but also with a Luer syringe *S* for pressure control, and with a reservoir *R*, the height of which was adjustable.

8 cm. of glass and rubber tubing (Fig. 1). Drainage of the chamber and the thistle tube was effected through a side arm midway between them. The solution was added by pouring it into the egg chamber from above. The outside electrode, itself, after

dipping into experimental solutions, could be flushed from a reservoir of saturated KCl and calomel.

It was found that variation in the magnitude of concentration potentials among different batches of eggs occurred, a striking fact in view of the negligible variation with age in the eggs from one female. A comparison of Tables 2, 3, 4, 5, and 8 shows, in KCl solutions at the same concentration range, slightly more than 100 per cent. variation of concentration potentials. Since conclusions drawn from these experiments are in every case based on relative rather than absolute values, however, the conclusions are not vitiated by this variation, because control experiments in any given study were always run within 24 hours on the same batch of eggs.

More difficult to cope with was the variation of P.D. across the same egg membrane, with time, and with successive washings in the same solution. This variation seemed to depend chiefly on differences in the thoroughness of washing; and secondarily on movements of the embryo within the egg, which disturbed the tightness of the electrical seal where the pipette penetrated the chorion. Probably in the brief time occupied by most of the experiments (less than half an hour for each egg), the factor of chorion permeability, which is discussed in connection with the experimental results of Table 1, was not important. The egg was washed with fresh solution after each reading until two successive readings were obtained which checked within about 5 per cent. A different experimental solution was then used.

The pH determinations required by the experiments were made with a quinhydrone electrode calibrated against standard buffer mixtures. The so-called neutral solutions were solutions of the pure salts made up in distilled water without the addition of any acid. Determinations of the pH of such solutions are of doubtful value, but a few which were made seemed to show that these solutions had a pH in the neighborhood of 5.4.

The experiments were carried out at temperatures which ranged from 20° to 26°, though they did not vary over more than 3° in the course of any one set of observations.

Experiments and Discussion.

One may recognize a membrane which is not equally permeable for all ions by the magnitude and direction of the potential differences to which it gives rise under certain sets of conditions. The dried collodion membrane has been shown by Michaelis and his colleagues (19, 22–27) to be of this sort. Out of the variety of criteria which these investigators have used to demonstrate the differential permeability of this and other membranes to ions, two were chosen for use with the *Fundulus* chorion. The first was the application to the membrane in question of two different concentrations of the same electrolyte solution; the second, the application of like concentrations of two different electrolytes.

When a dried collodion membrane (which is permeable for cations, but hardly, if at all, for anions) separates two different concentrations of the same electrolyte solution, a concentration potential results of such polarity that the dilute solution is positive with respect to the more concentrated solution. For any given electrolyte two features of such a concentration potential are of interest, namely, the sign, which is dependent on the greater permeability for cations, and the magnitude, which is a function of the degree of difference between the cation and the anion permeability. In the best dried collodion membranes the magnitude of the concentration potential with an electrolyte of univalent ions is close to the maximum theoretically possible (19) with a membrane perfectly impermeable for anions, but permeable for cations.

When a single egg was exposed to a series of dilutions of a KCl solution ranging by tenfold steps from M/2 to M/20,000, a series of concentration potentials was obtained (Table 1). In M/2 KCl (which is approximately isomotic with sea water) the outside solution was usually slightly negative with respect to the contents of the egg. All the more dilute solutions were positive with respect to the egg. The more dilute the solution, the greater was the degree of this positivity. The sign of these concentration potentials, therefore, is indicative that the chorion is more permeable for cations than for anions. The direction of polarity in M/2 KCl is such as would be obtained if the subchorionic fluid of the egg were slightly less concentrated with respect to electrolytes than this solution, though more concentrated than the rest.

The magnitude of the negative P.D.'s, however, is too small for much emphasis to be laid upon this point.

Table I.

Concentration Series.

Potential differences between the inside and the outside of 5 eggs immersed in a succession of KCl solutions of the indicated concentrations. The sign of the outside solution was positive except where the negative sign occurs.

No.	Age in Days.	$\frac{M}{2}$.	$\frac{M}{20}$.	$\frac{M}{200}$.	$\frac{M}{2,000}$.	$\frac{M}{20,000}$.	$\frac{M}{2,000}$.	$\frac{M}{200}$.	$\frac{M}{20}$.	$\frac{M}{2}$.
1	5	0.8 −	0.3	10.6	55.9	107.7	46.7	3.7	0.8 −	0.8 −
2	7	2.4	5.3	18.0	55.0	99.2	56.0	8.8	0.7	0.4
3	7	0.3 −	3.1	14.5	49.7	95.7	58.0	17.8	1.2	0.3
4	7	0.1 −	0.7	6.7	37.9	91.9	53.6	8.2	0.6	0.3 −
5	6	1.9 −	1.6	13.1	44.8	84.3	50.9	10.7	2.4 −	1.9 −

It will be observed in Table 1 that the concentration potentials (*i.e.*, the changes of P.D. between any two successive readings) increase markedly with dilution. Those from the ascending part of the series are shown in Table 2. The average value rises from

Table II.

Variation of Concentration Potentials with Dilution.

Concentration potentials obtained by subtracting adjacent values in the ascending parts of the series shown in Table I. The more dilute solution was positive.

No.	$\frac{M}{2} - \frac{M}{20}$.	$\frac{M}{20} - \frac{M}{200}$.	$\frac{M}{200} - \frac{M}{2,000}$.	$\frac{M}{2,000} - \frac{M}{20,000}$.
1	1.1	10.3	45.3	51.8
2	2.9	12.7	37.0	44.2
3	3.4	11.4	35.2	46.0
4	0.8	6.0	31.2	54.0
5	3.5	11.5	31.7	39.5
Average	2.3	10.4	36.1	47.1

one negligibly small in the interval between M/2 and M/20 to 47.1 mv. between M/2,000 and M/20,000, which is of the order of magnitude of the maximum of 58 mv. theoretically possible for a membrane completely impermeable for anions. In this be-

havior the egg membrane resembles a "poor" or large pored dried collodion membrane (24, 25). "Good" dried collodion membranes give very nearly the theoretical maximum even in fairly concentrated solutions.

An important feature of such a concentration series is the reversibility and reproducibility of the P.D.'s obtained when an egg is exposed to the same series of concentrations in the reverse order. In the experiments summarized in Table 1 each reading was obtained after the egg had been washed in at least six changes of the solution, but more prolonged washing, until two readings were obtained that checked, was not attempted. Such a series required for its completion approximately 45 minutes, with an exposure of about 5 minutes to each solution. The values obtained in this way were, therefore, probably lower on the ascent, and higher on the descent, than the definitive values for the concentrations in question; but the similarity shown by the experimental results for ascent and descent makes it appear probable that they were actually close approximations to these definitive values. The fact that in most cases the descending value was higher than the ascending one in the same solution indicates that dilution of the subchorionic fluid did not occur during the experiment, since this would have diminished the second P.D. observed.

When the concentration potentials obtained across a membrane with solutions containing ions of different valence are compared, further information is furnished as to the differential permeability of the membrane. If it is impermeable for anions but permeable for cations, concentration potentials across it are independent of the valence of the anion in the electrolyte solutions used, but are, theoretically, halved by doubling the valence of the cation (19).

The concentrations M/200–M/2,000 [3] were arbitrarily chosen as a test range for the study of concentration potentials with salts yielding bivalent anions or cations. It was found that the concentration potential for a bivalent anion, SO_4, was identical with that for the univalent Cl. Table 3 shows typical results on 5 eggs in KCl and 5 others in K_2SO_4. There was less than 1 mv. of difference between the averages. This failure of the valence of

[3] The concentrated solution was always used before the dilute.

the anion to affect the concentration potential is another point of resemblance between the *Fundulus* chorion and the dried collodion membrane, and is additional evidence that the chorion is at least relatively impermeable for anions.

Table III.

Effect of Anion Valence on Concentration Potentials.

Concentration potentials were measured between M/200 and M/2,000 solutions of KCl and K_2SO_4, a different egg being used for each measurement. The more dilute solution was positive.

KCl.	K_2SO_4.
32.4	39.3
32.7	33.5
33.7	29.3
30.3	33.6
38.4	36.2
Average 33.5	34.4

On the other hand, when it was the cation whose valence was doubled, the concentration potential across the *Fundulus* chorion was approximately halved. The effect was shown with $CaCl_2$, $MgCl_2$, and $BaCl_2$ (Table 4). The behavior of the chorion is

Table IV.

Effect of Cation Valence on Concentration Potentials.

Concentration potentials were measured between M/200 and M/2,000 solutions of KCl, $CaCl_2$, $BaCl_2$, and $MgCl_2$. The effects of Ba and Mg were not studied in the same batch of eggs with Ca, and are therefore exhibited with a different set of controls in KCl. In every case the more dilute solution was positive.

	KCl.	$CaCl_2$.	KCl.	$BaCl_2$.	$MgCl_2$.
	39.0	17.1	18.8	6.4	9.9
	31.6	16.3	23.7	15.4	13.0
	29.4	14.5	21.3	9.7	6.8
	30.9	13.8	22.7	9.8	14.1
	33.5	17.0	19.5	6.6	11.9
Average . .	31.6	15.7	21.2	9.6	11.1

precisely what would be predicted by the simplest theory for an ideal membrane. It is different, however, from the behavior of a

dried collodion membrane, which with $CaCl_2$ gives no concentration potential at all, because it happens to be impermeable for Ca as well as for Cl (30).

Most of the experiments with valence effects of cations were made with equimolecular solutions of the different salts, *i.e.*, solutions containing equal numbers of cations. In a few experiments equivalent solutions, containing equal numbers of anions were used for comparison. Thus, as an example of the latter type of experiment, the concentration potential for M/400–M/4,000 $CaCl_2$ instead of M/200–M/2,000 $CaCl_2$ was compared with that for M/200–M/2,000 KCl. As might have been expected, the results obtained in this way (Table 5) did not differ greatly from those in which equimolecular solutions were employed.

Table V.

Effect of Cation Valence on Concentration Potentials. Choice of Concentration Range.

Concentration potentials with KCl and $CaCl_2$ were compared in both equivalent and equimolecular solutions. Two sets of controls in KCl appear because the $CaCl_2$ experiments were made on different days.

	KCl, $\frac{M}{200} - \frac{M}{2,000}$.	$CaCl_2$, $\frac{M}{400} - \frac{M}{4,000}$.	KCl, $\frac{M}{200} - \frac{M}{2,000}$.	$CaCl_2$, $\frac{M}{200} - \frac{M}{2,000}$.
	31.5	20.7	32.7	14.2
	42.5	19.4	44.2	18.4
	23.6	9.4	40.3	11.7
Average...	32.5	16.5	39.1	14.8

Still further evidence for the relative impermeability of the chorion for anions was obtained when the second test was applied: *i.e.*, the exposure of the egg to a series of salt solutions alike in concentration but differing in composition. Against the dried collodion membrane equal concentrations of different salt solutions containing the same cation are isoelectric; but when the anion is the common ion, they give rise to P.D.'s differing in magnitude in the same order as the classic mobility values of the cations used (19).

Tables 6 and 7 show the P.D. in mv. between the inside and outside of eggs each of which was exposed successively to all the

solutions shown in that table. The values obtained with different eggs in the same solution are of no interest, except to show the fair constancy of the material; but the values obtained with a

Table VI.

P.D. against Different Anions of the Same Concentration.

P.D.'s between the solution and the egg contents are given from six typical experiments in each of which one egg was exposed to all of the following salts of K. Variation of the order in which the solutions were used had no effect.

No.	Cl.	Br.	I.	SCN.	Acetate.	NO_3.
1	46.4	40.8	44.0	44.1	42.1	44.2
2	48.6	43.9	49.2	47.5	44.6	45.5
3	50.6	45.6	50.9	48.8	44.0	46.9
4	52.6	45.6	49.7	50.0	50.8	45.5
5	41.7	42.1	43.5	43.2	41.0	41.5
6	42.1	42.4	42.0	40.0	37.9	40.9
Average	47.0	43.4	46.5	45.6	43.4	44.1

given egg in different solutions are of interest, and the averages of these indicate the general trend of the effects. It will be seen from Table 6 that there is no difference of potential which may be

Table VII.

P.D. against Different Cations of the Same Concentration.

Single eggs were exposed to a succession of M/2,000 solutions of the following chlorides. P.D.'s across the chorion from 5 typical experiments are given. Variation of the order in which the solutions were used had no effect.

No.	Li.	Na.	K.	Rb.	Cs.
1	61.3	55.0	54.0	38.1	31.9
2	47.8	48.6	48.7	28.7	29.8
3	59.3	49.3	48.1	33.4	32.1
4	58.8	58.4	49.2	37.7	31.7
5	63.4	57.9	56.1	37.8	38.2
Average	58.1	53.8	51.2	35.1	32.7

considered significant among the K salts of Cl, Br, I, SCN, acetate, and NO_3. But when the anion is kept constant and the cation is varied, a series appears in which the cations, Li, Na, K,

Rb, and Cs, are arranged in the order of their classic mobility values, LiCl being positive to all the other chlorides used, and CsCl negative. It is interesting to note that while the order for the series is correct, the values obtained with Li, Na, and K are very close together, while those with Rb and Cs fall in a separate group at some distance from the others; whereas the most pronounced break in the mobility values for free diffusion is not between K and Rb, but between Na and K. The results of this experiment may be interpreted to mean not only that the membrane possesses differential permeability for ions of opposite sign, but also that differences are present, though to a lesser degree, in the permeability for univalent cations. Of the ions studied, Cs appears to penetrate most readily, and Li least readily.

The results of these experiments, in which the dilution, valence, and chemical identity of the different ions in the solution applied to the membrane has been systematically varied, may be confidently interpreted to mean that in approximately neutral solutions the chorion is more permeable for cations than for anions. This conclusion may be used as the basis of the following partial interpretation of Loeb and Cattell's results (16), though the complete explanation must be impossible until the electrical properties of the ectoderm have been studied in addition to those of the chorion.

If K, in order to stop the heart-beat of a *Fundulus* embryo, must penetrate both chorion and ectoderm, then recovery can be effected only by exit of K through that double membrane. The escaping K must either be accompanied by anions in an equivalent amount or exchanged for cations from the outside solution. Most of the movement of the cations must be accomplished in the second way because, as the present experiments indicate, anions pass with difficulty across the chorion. Therefore K escapes much more slowly into distilled water than into a solution, whether of salt or of acid.[4] The same explanation holds for the retardation of K penetration into eggs which have been soaked for 24 hours in distilled water. In these, Armstrong (2) has found that the sub-

[4] McClendon (17) gave this explanation briefly for the failure of Mg to escape from *Fundulus* eggs into distilled water; but when he reported later (18) that Mg also failed to escape into Van't Hoff's solution, he offered a different explanation, not only for this, but for the former result.

chorionic fluid has the pH of the surrounding medium. It seems probable, therefore, that the subchorionic fluid in these eggs has been largely replaced by distilled water.

The results of other experiments by Loeb are not so easily related to the the theory of differential permeability of the chorion for ions; for instance, the observation (11, 12, 13, 16) that K enters unwashed eggs more readily from a pure KCl solution than from a mixture of KCl with some other electrolyte. The additional electrolyte in this case may perhaps alter the degree of differential permeability of the membrane. This possibility will be mentioned in another connection.

Although measurements of P.D. yield direct evidence for the relative numbers of ions of opposite sign penetrating the membrane, the absolute numbers are not so directly indicated. Results of the type obtained would be possible under several states of ion permeability. For example, ions of both signs may traverse the chorion fairly readily, but at different rates; or both may fail almost completely to penetrate, though having sufficiently different penetrating tendencies to yield a P.D.; or, finally, cations may pass without anions. The results of several investigators working with different methods and criteria make it appear that the chorion is at least somewhat permeable for cations. Loeb (11, 19) found the eggs permeable for a dye cation, neutral red. Armstrong (3) showed that when heart standstill was brought about by excess K or acid in the surrounding solution there was no difference between the kind of effect on naked embryos and on embryos surrounded by a chorion. Bodine (4) has reported that the only difference under such circumstances is one of time. The chorion is probably permeable for all ions applied in sufficiently concentrated solutions or over long enough periods of time; and for cations even in dilute solutions, provided that electrical neutrality can be maintained by an exchange with other cations.

Several studies by Loeb (8, 9, 10, 14, 15) and one by Armstrong (3) on salt antagonism for acid penetration into *Fundulus* eggs, as well as a few experiments reported by Loeb (10) and Loeb and Cattell (16) on the opposite, namely, acid antagonism for salt penetration, have been of considerable interest; yet their

mechanism is imperfectly understood. It was thought, therefore, that an investigation of potential differences across the membranes of eggs in acid solutions might throw some light on this problem. Accordingly, the first of the tests used on eggs in neutral solutions was made, *i.e.*, the application to the chorion of different concentrations of the same electrolyte solution, with the modification that the solutions were brought to a desired pH value by the addition of an appropriate acid. The sign and magnitude of the concentration potentials were studied, and a comparison was made of the effects on them of di- and uni-valent ions.

When an egg was exposed in succession to two solutions of KCl, one M/20, the other M/200, to both of which sufficient HCl had been added to bring the pH to 3.0, a concentration potential was obtained with the polarity the reverse of that found in neutral solutions; that is, the more dilute solution was negative to the more concentrated. The experimental results given in the middle column of Table 8 deal with 5 eggs, each of which was studied in

Table VIII.

Reversal of KCl Concentration Potentials with Increase in the H Ion Concentration.

Concentration potentials were measured between M/20 and M/200 solutions of KCl to which sufficient HCl had been added to give the desired pH. A different egg was used for each measurement. Sign of the dilute solution was positive except where the negative sign occurs.

pH.				
2.0	2.5	3.0	3.5	4.0
0.5	1.7 —	5.8 —	4.7 —	38.2
1.3	9.1 —	8.9 —	3.2 —	34.0
0.7	7.4 —	9.6 —	18.0	46.7
2.1 —	9.8 —	10.0 —	3.4 —	30.6
4.4	11.8 —	12.6 —	9.8	37.7

Controls.

Concentration potentials previously shown by the same 25 eggs between M/20 and M/200 solutions of pure KCl.

18.4	23.1	20.9	25.6	35.3
25.8	26.5	22.0	28.4	38.0
22.6	25.4	——	30.9	39.7
21.1	24.3	——	30.8	40.4
16.3	25.7	25.5	33.0	36.8

pure solutions of KCl as well as in KCl at pH 3.0. The concentration potential values obtained at pH 3.0, while smaller than those in pure KCl, were manifestly in the opposite direction.

The range of salt concentrations chosen for these experiments with acid solutions was higher than in the experiments with neutral solutions because it was desired to study the concentration effect of the salt itself, rather than that of total electrolyte content. The complexity introduced by the use of a solution containing two electrolytes is simplified somewhat if the salt is relatively concentrated as compared with the acid. For the same reason, a pH of 3.0 was chosen for the subsequent comparison of KCl with other salts, rather than one of 2.0 or 2.5, where the acid would be a more significant element in the concentration. The addition of HCl in equal amounts to both concentrated and dilute solutions reduces the ratio of their concentrations with respect to total electrolyte. At a pH of 3.0 the HCl has approximately a concentration of 0.001 M. Thus the total electrolyte concentrations of the two solutions compared were 0.051 M and 0.006 M, respectively, and their ratio was 8.5 instead of 10. At pH, 2.5, their ratio was approximately 6.6, and at pH, 2.0, 4. The order of these relationships is not altered if activities are substituted for concentrations. The concentration potentials to be expected in acid solutions must therefore be less, in accordance with this reduction of the concentration ratios.

Despite the disadvantages of the more acid solutions (both because of their disturbance of the concentration ratio, and also because of their destructive effect on the membrane, to be referred to later), a study of concentration potentials was made at a series of different pH values in order to determine, if possible, the point at which reversal occurs. Table 8 also shows the results of these experiments, presenting under each of five pH values the concentration potentials obtained in both acid and neutral solutions with the same eggs. At a pH of 4.0, the concentration potentials were found to be in the same direction as the control values, and very slightly smaller. At 3.5, all were much reduced and 3 out of 5 were reversed. At 3.0 and 2.5 all were reversed and of considerable magnitude, but at 2.0 there was scarcely any concentration potential in either direction. Apparently the reversal point

lies slightly above a pH of 3.5, probably in the neighborhood of 3.7.

The marked reduction of the concentration potentials at pH 2.0 was in part to be expected because of the reduction of the concentration ratio which the addition of acid brings about. But it seems probable that at this lower extreme of the pH range there is also a destructive effect of the acid on the membrane which occurs too rapidly to permit detection of the characteristic potential differences. In harmony with this suggestion are two instances, shown in the column for pH, 3.0 in which, contrary to the procedure with the other eggs, the acid solutions were used before the neutral ones; with the result that no concentration potential was obtained in the subsequent control experiment. Apparently even this dilution of acid, in a period of 10 or 15 minutes, exercised some irreversible destructive effect on the membrane which abolished its differential permeability for ions.

The acid reversal of the sign of concentration potentials in KCl suggested the possibility that acidity might operate also to reverse the valence effect on concentration potentials, so that $CaCl_2$ would give values equal to those obtained with KCl, and K_2SO_4 values less by half. To test this theory, 5 eggs were studied in $CaCl_2$ solutions M/20 and M/200 at pH 3.0, and 5 other eggs in equimolecular solutions of KCl at the same reaction. It was found (Table 9), as had been expected, that concentration potentials with

TABLE IX.

EFFECT OF CATION VALENCE ON CONCENTRATION POTENTIALS AT PH 3.0.

Concentration potentials were measured between M/20 and M/200 solutions of KCl and $CaCl_2$, all brought to a pH of 3.0 with HCl. A different egg was used for each measurement. Sign is that of the dilute solution.

KCl.	$CaCl_2$.
14.5 —	23.3 —
13.1 —	13.8 —
13.4 —	19.2 —
7.6 —	20.6 —
4.4 —	19.0 —
Average 10.6 —	19.2 —

$CaCl_2$ at this pH were also reversed and were not smaller than those obtained with KCl. Indeed they were considerably larger.

(The difference was somewhat greater when they were compared in equivalent concentrations.) On the other hand, K_2SO_4, gave concentration potentials of the same sign as at neutrality (Table 10). The reversal point with K_2SO_4, if one exists, must be at a pH lower than 3.0.

TABLE X.

EFFECT OF ANION VALENCE ON CONCENTRATION POTENTIALS AT PH 3.0.

Concentration potentials were measured between M/20 and M/200 solutions of KCl and K_2SO_4 adjusted to a pH of 3.0 with HCl and H_2SO_4 respectively. A different egg was used for each measurement. The dilute solution was positive except where the negative sign occurs.

KCl.	K_2SO_4.
11.2 —	22.3
10.2 —	13.6
4.4 —	28.8
12.1 —	15.0
16.6 —	8.9
Average 10.9 —	17.7

Such results as these acid effects on concentration potentials are not obtained across dried collodion membranes (19). An inversion of concentration potentials with increased acidity has been reported for membranes of other materials by Mond (27), Fujita (6), Rein (29), and Amberson and Klein (1), but not for any membranes across which chemically controlled diffusion experiments have also been made. Nevertheless, logically interpreted, the reversal of concentration potentials across the *Fundulus* chorion in KCl and $CaCl_2$ solutions seems to mean that in acid solutions of those salts the chorion is more permeable for anions than for cations. The reversal point, then, is the pH where the membrane is equally permeable for ions of both signs.

The application of these results to interpretation of the studies by Loeb and by Armstrong of the antagonistic action between salt and acid is very difficult. More electrical experiments are needed, testing the effect of a greater variety of salts, concentrations, and pH values; but the direction in which such further experiments will be useful may be indicated here. We may assume that the hindrance either of KCl or of acid penetration involves a decrease in the permeability of the membrane for cations. But, as has already been pointed out, the actual number of ions

of either sign which penetrates cannot be directly determined from measurements of P.D. Equal ion permeability at the reversal point may be due to a diminution in permeability for cations, or to an increase in that for anions, or to both. So far, then, as acid antagonism for salt penetration may be correlated with the present results, Loeb's experiments (10) give more than they receive of illumination: because the fact of acid antagonism for salt penetration implies that the reversal point in the electrical experiments is produced mainly in the first way, *i.e.*, by diminution in permeability for cations.

At least two features of the results secured have not been explained. These are the facts that concentration potentials with $CaCl_2$ exceed those with KCl at pH 3.0, and that concentration potentials with K_2SO_4 are not reversed at all at that reaction. These facts seem to mean that the properties of the chorion are not due entirely to the pH of the medium, but depend also on its salt content. They may perhaps furnish a clue to the way in which salt antagonism for acid penetration, and perhaps also for the penetration of other salts, may be brought about. However, it should be remembered that the simple interpretation of the potential differences obtained across the chorion in terms of its differential permeability for ions does not explain the more fundamental question of how such differences in its ion permeability are produced.

Summary.

1. Potential differences were measured across the chorion of single eggs of *Fundulus heteroclitus*. The chorion was shown by three lines of evidence to be more permeable for cations than for anions:

a. Concentration potentials were of such a sign that the dilute solution was positive to the concentrated.

b. Concentration potentials with K salts of divalent and univalent anions were equal, whereas concentration potentials with chlorides of divalent cations were about half of those with K.

c. Equal concentrations of different anions were eqipotential against the egg, whereas equal concentrations of different cations gave various potential differences whose magnitudes were in the

same order inverted as the mobilities of those cations in free diffusion.

2. The difference between the permeability of the chorion for anions and its permeability for cations increased with dilution of the solution in which the egg was immersed.

3. In KCl and $CaCl_2$ solutions the ratio of chorion permeability for anions to permeability for cations increased with the H ion concentration, and was inverted with sufficiently increased acidity.

4. The pH of the reversal point, where permeability for anions was equal to permeability for cations, depended on the salt used. For KCl in the concentrations used it lay in the neighborhood of 3.7.

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